This figure "FIG1.png" is available in "png" format from:

http://arXiv.org/ps/q-bio/0510048v1

Trajectory:	[RC]	[P1]	[P2]	[DU]	[RCS]
Total Time (ns):	102.6	65.0	83.6	80.0	145.0
Events (ns):					
S^*	37.1	45.9	27.0	12.6	34.0
$R^*(4,8)$	35.2	46.6	21.4	9.6	30.8
$R^*(2,8)$	7.6	8.1	5.9	0.4	19.0
$R^*(3,8)$	5.9	14.7	1.7	1.7	18.0

Table 1: Table of total accumulated times of events in each of the five trajectories.

This figure "FIG2.png" is available in "png" format from:

http://arXiv.org/ps/q-bio/0510048v1

Overlaps:	$S^* \cap R^*(4,8)$		$R^*(4,8) \cap SB$		$R^*(4,8) \cap R_g^*$		$R^*(2,8) \cap R^*(3,8)$	
	S^*	$R^*(4,8)$	$R^*(4,8)$	SB	$R^*(4,8)$	R_g^*	$R^*(2,8)$	$R^*(3,8)$
Trajectory:								
[RC]	91.9	96.7	34.9	90.8	72.3	79.7	0.0	0.0
[P1]	97.1	95.6	40.6	84.9	95.9	85.8	5.3	2.9
[P2]	76.0	95.8	1.2	3.4	73.9	45.7	0.0	0.0
[DU]	72.6	95.7	4.3	19.7	68.1	41.3	5.7	1.4
[RCS]	85.7	94.6	54.1	70.4	81.1	60.2	70.1	73.9

Table 2: Table of percentage of overlap (columns) between pairs of events per trajectory (rows).

This figure "FIG3.png" is available in "png" format from:

http://arXiv.org/ps/q-bio/0510048v1

Solvent and mutation effects
on the nucleation of amyloid β-protein folding

Luis Cruz*¶, Brigita Urbanc*, Jose M. Borreguero*†,
Noel D. Lazo‡, David B. Teplow‡ and H. Eugene Stanley*¶

* *Center for Polymer Studies and Department of Physics, Boston University, Boston, MA 02215.*
† *Center of Excellence in Bioinformatics, SUNY at Buffalo, NY 14203.*
‡ *Department of Neurology, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095.*

Classification: Physical Sciences: Applied Physical Sciences and Biological Sciences:

Biophysics.

22 text pages (text, references, and legends), **2** tables, and **3** figures.

250 words in abstract and **46,722** total number of characters in the paper.

¶**Corresponding Authors: L. Cruz and H.E. Stanley**

Center for Polymer Studies, Department of Physics,

590 Commonwealth Ave, Boston University,

Boston, MA 02215

Phone: 617-353-2617

Fax: 617-353-3783

Email: ccruz@bu.edu and hes@bu.edu

Abbreviations: Aβ, amyloid β-protein; AD, Alzheimer's disease; DMD, discrete

molecular dynamics; HB, hydrogen bond; HCHWA-D, hereditary cerebral hemorrhage with

amyloidosis–Dutch type; MD, molecular dynamics; SASA, solvent accessible surface area;

SB, salt bridge.

Abstract

Experimental evidence suggests that the folding and aggregation of the amyloid β-protein (Aβ) into oligomers is a key pathogenetic event in Alzheimer's disease (AD). Inhibiting the pathologic folding and oligomerization of Aβ could be effective in the prevention and treatment of AD. Here, using all-atom molecular dynamics simulations in explicit solvent, we probe the initial stages of folding of a decapeptide segment of Aβ, Aβ_{21-30}, shown experimentally to nucleate the folding process. In addition, we examine the folding of a homologous decapeptide containing an amino acid substitution linked to hereditary cerebral hemorrhage with amyloidosis–Dutch type, [Gln22]Aβ_{21-30}. We find that: (i) when the decapeptide is in water, hydrophobic interactions and transient salt bridges between Lys28 and either Glu22 or Asp23 are important in the formation of a loop in the Val24–Lys28 region of the wild type decapeptide; (ii) in the presence of salt ions, salt bridges play a more prominent role in the stabilization of the loop; (iii) in water with a reduced density, the decapeptide forms a helix, indicating the sensitivity of folding to different aqueous environments; (iv) the "Dutch" peptide in water, in contrast to the wild type peptide, fails to form a long-lived Val24–Lys28 loop, suggesting that loop stability is a critical factor in determining whether Aβ folds into pathologic structures. Our results are relevant to understand the mechanism of Aβ peptide folding in different environments, such as intra- and extracellular milieus or cell membranes, and how amino acid substitutions linked to familial forms of amyloidosis cause disease.

Keywords: molecular dynamics, amyloid β-protein, Alzheimer's disease, nucleation, protein folding.

1 Introduction

The amyloid cascade hypothesis, first proposed in the early 1990's, posits that the deposition of amyloid fibrils is the seminal event in the pathogenesis of Alzheimer's disease (AD) [1, 2]. However, recent biophysical, biological, and clinical data indicate that the formation of oligomeric assemblies much smaller than fibrils may be a key pathologic event [3–10]. This paradigm shift suggests an attractive therapeutic approach–prevent or disrupt the assembly of Aβ monomer into toxic oligomers. To do so requires an understanding of the molecular dynamics involved in the transition from non-toxic monomeric to toxic oligomeric states.

Elucidation of the initial events in Aβ monomer folding and assembly is complicated by the solvent dependence of the process [11]. Monomeric Aβ is largely helical in a membrane or membrane-mimicking environment such as ionic detergents [12–15]. In contrast, Aβ monomers in aqueous solution show negligible α-helix or β-sheet content [11, 16, 17]. Studies of Aβ_{10-35}-amide at micromolar concentrations in water show a pH-dependent folding transition in which the conformation is not helical but contains several turns and at least two short strands [18]. Solution-state NMR combined with diffusion-ordered spectroscopy indicate that variation of the anionic strength in the buffer shifts the equilibrium between monomeric and oligomeric states, possibly allowing for the stabilization of intermediate structures [19]. Molecular dynamics (MD) studies of Aβ_{16-22} monomer in aqueous urea solution show that increasing the concentration of urea promotes a transition from a compact random coil to β-strand structures [20].

Aβ folding and oligomerization are also influenced significantly by amino acid structure at specific sites. The dipeptide Ile41-Ala42 at the C-terminus of Aβ_{1-42} is responsible for the different biophysical behaviors of Aβ_{1-42} and Aβ_{1-40} [21, 22]. Oxidation of Met35 disrupts

Aβ_{1-42} oligomer formation but does not affect Aβ_{1-40} oligomer formation, consistent with the possibility that there are structural differences between oligomers of the two alloforms involving Met35 and neighboring residues [23]. Simulations of the oligomerization of Aβ_{1-40} and Aβ_{1-42} [24, 25] using discrete MD (DMD) with implicit solvent [26–29] are consistent with *in vitro* data, yield new structural predictions, and offer a plausible mechanistic explanation of the Met35 oxidation experiments [25]. MD studies show that the presence of glycines induce a transition from an α-helix to a β-strand conformation in Aβ_{1-40} monomers in aqueous solution [30]. Simulation studies of the Dutch [Gln22]Aβ_{10-35} peptide in water indicate that interactions between the peptide and the solvent are stronger in the wild type than in the mutant peptide [31].

Recent limited proteolysis experiments on Aβ_{1-40} and Aβ_{1-42}, under conditions favoring oligomerization, identify in both peptides a protease-resistant segment, Ala21–Ala30. The homologous decapeptide Aβ_{21-30} shows identical protease resistance [32]. Structure calculations based on distance constraints from proton solution-state NMR of Aβ_{21-30} reveal a turn structure at Val24–Lys28. Lazo *et al.* [32] postulate that this structure nucleates the intramolecular folding of the Aβ monomer and that partial unfolding of the Ala21-Ala30 region may be necessary for the subsequent fibrillization of Aβ. The observations on full-length Aβ and on the Aβ_{21-30} decapeptide are consistent with previous work showing that peptide fragments containing the folding nuclei of globular proteins are, by themselves, structured [33]. Furthermore, the structures found in the isolated folding nuclei are similar to those found in the full-length proteins [33]. MD simulations of the folding nucleus, therefore, provide insights into the earliest events in the folding of the full-length protein. Borreguero *et al.* recently used DMD with implicit solvent and a united-atom protein model to simulate folding of the putative Aβ folding nucleus Aβ_{21-30} [34]. The united-atom peptide model

considers explicitly all protein atoms except hydrogen. Important findings in Borreguero *et al.* [34] are: (i) the existence of a loop that is stabilized by hydrophobic interactions in the Val24–Lys28 region; (ii) a high degree of flexibility in the termini; and (iii) electrostatic interactions between the charged groups of Glu22, Asp23, and Lys28 that modulate the stability of the folded structure.

Here we test whether the stability of the Val24–Lys28 loop described by Borreguero *et al.* [34] persists in simulations that consider an explicit solvent (all atoms are included in the simulation). In addition, we determine the effects of solvent alterations on the folding dynamics and investigate the changes in the dynamics caused by amino acid substitutions. To this end, we present results of long-time, all-atom MD simulations of $A\beta_{21-30}$ in explicit water. We also study the dynamics of the monomer containing the Dutch $[Gln22]A\beta_{21-30}$ mutation. This mutation leads to hereditary cerebral hemorrhage with amyloidosis–Dutch type (HCHWA-D), characterized by extensive deposition of $A\beta_{1-40}$ in arterioles and small cerebral vessels but only limited senile plaque formation. *In vitro* studies indicate that the Dutch $A\beta$ has a greater tendency to form protofibrils and fibrils than does the wild type $A\beta$ [3, 35]. To investigate dynamical differences in the folding due to changes in the composition and density of the solvent, we study the system with solvated ions in normal density water and in water with a reduced density. Our results are consistent with previous results [34] and, in addition, reveal a helix conformation of the decapeptide when the encompassing water has a lower density than normal water. Simulations with solvated ionic atoms in the water illustrate how a different solvent composition can influence the role of salt bridges between the charged amino acids by strengthening the stability of the Val24–Lys28 loop. In addition, simulations of $A\beta_{21-30}$ containing the Dutch $[Gln22]A\beta_{21-30}$ mutation suggest that the experimentally-observed increased fibril formation of this peptide may be a consequence

of the increased number of aggregation-prone unpacked conformations.

2 Methods

MD Simulations. We perform long-time MD simulations of $A\beta_{21-30}$ monomer in water at normal and reduced density, normal density water with dissociated salt ions, and the Dutch [Gln22]$A\beta_{21-30}$ monomer in normal density water. We explicitly consider all atoms with potential energies given by the CHARMM-27 force-field [36] using the NAMD package [37]. We use the TIP3P [38] model for the water molecules. We use the NVT ensemble and confine the system in a box with periodic boundary conditions. We carry out the MD simulations at a constant temperature of $T = 283$K, corresponding to the *in vitro* experiments [32].

$A\beta_{21-30}$ has the primary structure Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala, where Glu22 and Asp23 are negatively charged and Lys28 is positively charged, while the Dutch mutation [39] substitutes Glu22 by Gln22 (neutral). Following [32], we block $A\beta_{21-30}$ with NH_3^+ and CO_2^- at the N- and C- termini, respectively. We generate five trajectories with the following initial conditions:

- [RC], wild type random coil conformation in normal density water;

- [P1] & [P2], wild type loop conformations from Lazo *et al.* [32] in water with reduced density (corresponding to Families I and II from Ref. [32], respectively);

- [DU], Dutch peptide in a random coil conformation in normal density water;

- and [RCS], wild type random coil conformation in salted water.

We choose these five trajectories because they give information about the dynamics of the wild type $A\beta_{21-30}$ in normal water and water with a reduced density, starting from ex-

perimentally postulated conformations ([P1] and [P2]) and a control [RC]. These are then compared with the Dutch [DU] and with the wild type peptide in a salted solvent [RCS].

We solvate each monomer by inserting it in the center of a previously-equilibrated cube of water molecules of side 43Å. This insertion deletes all water molecules overlapping or in close proximity ($<$2.4Å) to any of the monomer atoms, resulting in a system with about 2542 water molecules (see *Supporting Information* for details). To maintain system neutrality (for wild type only), we insert a single Na^+ ion far from the monomer. To obtain the "salted" system, we insert 25 dissociated molecules of NaCl, resulting in a system with 2399 water molecules. We carry out all the insertions and preparations of the systems using the VMD package [40]. We use the Particle Mesh Ewald method [41] to calculate the electrostatic interactions and a cut-off distance of 12Å for the direct electrostatic and van der Waals interactions. We use a timestep of 1 fs for all simulations and save configurations (monomer and water) every 2 ps.

We first minimize the energy of the system for 20,000 steps by applying the conjugate gradient algorithm that relaxes all atoms except the C_α atoms. Next, we release the C_α atoms and minimize again for another 20,000 steps. Then, we gradually heat the system in the NVT ensemble from 0 to 283K by harmonically constraining all C_α atoms for 100 ps. Following this step, and still constraining the C_α atoms, we then perform another 200 ps in the NPT ensemble at 283K, followed by a brief 50 ps NPT simulation with no constraints to generate the starting configuration for the production runs. Because of the way that we delete overlapping water molecules in the solvation step above, these NPT equilibration steps allow for water molecules to fill in holes left from the insertion of the decapeptide, thus shrinking the size of the system by about 1Å in all directions (see *Supporting Information* for details). For trajectories [P1] and [P2], we skip the NPT equilibration, not allowing

the system to shrink, thus creating a system in which the effective density of the water is reduced by about 7.5%. In Table 3 (*Supporting Information*) we list all of the thermodynamic quantities for each of the trajectories. Total simulation times for the production runs are 102.6 ns for [RC], 65 ns for [P1], 83.6 ns for [P2], 80.0 ns for [DU], and 145.0 ns for [RCS]. Although these simulation times are too small to study the totality of the folding process of the Aβ_{21-30}, they are long enough to exhibit characteristic elements of the dynamics of the folding for this decapeptide.

Structural Determinants. One of the most important quantities characterizing the structure of Aβ_{21-30} is the distance between atoms. The notation R(x, y) signifies the distance R between two specified atoms from amino acid x and amino acid y. The quantities we use to characterize the structure of Aβ_{21-30} as a function of simulation time are: (i) the distance between the two C$_\alpha$ atoms of Ala21 and Ala30 (denoted by R(1,10)) and Val24 and Lys28 (denoted by R(4,8)); the distance between Lys28(N$_\zeta$) and Glu22(C$_\delta$) (denoted by R(2,8)); the distance between Lys28(N$_\zeta$) and Asp23(C$_\gamma$) (denoted by R(3,8)), (ii) the radius of gyration R_g of the monomer given by $R_g^2 \equiv \sum_i m_i(|\vec{r_i} - \vec{r_c}|)^2 / \sum_i m_i$, where $\vec{r_c}$ is the center of mass, (iii) the combined solvent accessible surface area (SASA) [42] of the Val24 and Lys28 side chains (denoted by S), (iv) the hydrogen bond contact maps between amino acids, (v) the secondary structure propensity for each amino acid, (vi) the normalized scalar product D between the vector joining C$_\alpha$ and C$_\beta$ of Val24 and the vector joining C$_\alpha$ and C$_\epsilon$ of Lys28, and (vii) the number and type of hydrogen bonds (HB) between atoms in the decapeptide [40]. We calculate secondary structure propensity using the program STRIDE [43] available through the VMD package. A HB exists if the distance between donor and acceptor is ≤3Å and the angle θ between the donor–hydrogen–acceptor atoms is $160° \geq \theta \leq 200°$. If, in addition, two hydrogen-bonded atoms are of opposite charge, they form a salt bridge

(SB) [44].

The quantities defined above provide a broad qualitative and quantitative description of the structure of Aβ_{21-30}. In particular, the distance R(4,8) monitors possible formation of a loop involving the Val24–Lys28 region and close proximity of the hydrophobic parts of the Val24 and Lys28 side chains. The secondary structure propensity per amino acid determines general structure. A decrease of the combined SASA of two side chains indicates that the side chains are packed, reducing their contact with the solvent. A reduced normalized scalar product D indicates that the side chains of Val24 and Lys28 are constrained in their movements, implying that they either are bound to the backbone or to other side chains. A small radius of gyration R_g along with a reduced R(1,10) indicate folded conformations. Small values of R(2,8) or R(3,8) indicate the possible formation of salt bridges between Glu22 and Lys28 or Asp23 and Lys28, respectively. Finally, the contact maps along with the number and type of hydrogen bonds determine the nature and strength of interactions between different amino acids.

3 Results

Distances. In Fig. 1 we show results for R(2,8), R(3,8), R(4,8), and R_g as a function of time. As expected, these quantities fluctuate, but they also show "events" that last extended periods of time in which their values are substantially reduced and their fluctuations are uncharacteristically small. R(1,10) did not exhibit "events" in any of the trajectories, indicating that the termini are flexible (results not shown). The graph of S as a function of time has a close functional resemblance to R(4,8) (results not shown). This functional resemblance arises because whenever Val24 and Lys28 are in close proximity (R(4,8)< 6.5Å),

S is reduced from about 575Å^2 (separated and accessible to solvent) to 475Å^2.

We define "events" in Fig. 1 by: (i) R*(4,8), in which R(4,8) < 6.5Å (Val24–Lys28 loop); (ii) R*(2,8) or R*(3,8), in which R(2,8) or R(3,8) < 4.2Å (salt bridges); (iii) S*, in which $S < 525\text{Å}^2$ (Val24 and Lys28 side chain packing); and (iv) R_g^*, in which R_g < 6.5Å (peptide packing). We classify (i) and (iii) as hydrophobic events and (ii) as electrostatic events with the formation of salt bridges (SB).

Events within a trajectory occur at different times and time intervals (Fig. 1). By adding up the duration in time of these events, we obtain the total event time per trajectory (Table 1). Values in Table 1 are reported in nanoseconds rather than as percentages because the total simulation times are too short to obtain statistically significant percentage results. From these data, we see that S* and R*(4,8) (hydrophobic events) total times in each trajectory are comparable. We also see that hydrophobic events last for much longer periods of time than SB (R*(2,8) and R*(3,8)) events.

We next determine the amount of temporal overlap between pairs of events (Table 2), helpful for establishing correlations between events. We define the percentage of overlap for event X between two events X and Y as the time that X and Y overlap divided by the total duration of event X, multiplied by 100. In Table 2, we divide each overlap into two subcolumns, one for each of the events. In the first overlap column ($S^* \bigcap R^*(4,8)$), we observe that in all trajectories the S^* and R*(4,8) events are correlated (high % of overlap), indicating that proximity of Val24 and Lys28 is linked to an energetically favorable hydrophobic interaction. In the next column, the values for the overlap between the R*(4,8) and the SB events (R*(2,8) and R*(3,8) taken together) demonstrate that, except for trajectories [P2] and [DU], the SB events mostly occur conditional on an existing R*(4,8) event. Trajectory [P2] does not allow for this overlap because of its different secondary structure

and HB contacts (*Supporting Information*). In trajectory [DU] the lack of a negative charge in the Gln22 that effectively eliminates all $R^*(2,8)$ events might be key to the lack of $R^*(4,8)$ and SB overlaps. Thus, the prominent overlaps of SB with $R^*(4,8)$ suggest that interactions between amino acids in positions 22, 23, and 28 in the wild type may be intermediary (or necessary) for extended $R^*(4,8)$ events to exist.

Next, examining $R^*(4,8) \cap R_g^*$ we observe that for most trajectories there is a high degree of correlation between the $R^*(4,8)$ and R_g^* events, indicating that the decapeptide has a smaller effective size whenever Val24 and Lys28 are in close proximity. Finally, in the last column ($R^*(2,8) \cap R^*(3,8)$) we see that the formation of the $R^*(2,8)$ and $R^*(3,8)$ salt bridges is mutually exclusive (except in trajectory [RCS]). This result indicates that while Glu22 (Asp23) interacts with Lys28, Asp23 (Glu22) interacts with other amino acids or water. In contrast, in trajectory [RCS] the large overlap between the two SB events suggests that the ions in the solvent facilitate simultaneous formation of both SBs possibly by disrupting properties of the water molecules surrounding the decapeptide.

Secondary Structure and Packing of Val24 and Lys28. In Fig. 2 we show the time evolution of the secondary structure propensity of each amino acid for all the trajectories. As with other structural determinants, there is a high degree of correlation between these secondary structures and other events. Typically, $R^*(4,8)$ events are correlated with increased turn propensities that span at least the Gly25–Lys28 region (Fig.4, *Supporting Information*). In Fig. 2 [P1], the π-helix correlates with lowered values of all of the R(2,8), R(3,8), and R(4,8) distances as well as with a very compact conformation with the lowest R_g (from Fig. 1). We observe a similar behavior in trajectory [P2] during the helix formation in the later part of the trajectory (75 to 80 ns). The helices in [P1] and [P2] are both formed under a pre-existing $R^*(4,8)$ event (compare R(4,8) from Fig. 1 with Fig. 2). We note that the prominent

helices in [P1] and [P2] both occur in the reduced density water environments whereas in the trajectories with normal density water there is no observable helix. In trajectory [DU], the row corresponding to Gly25 (label "25" on the y-axis) is less populated (turn) than the others, suggesting two turn regions separated at Gly25 (see Fig. 4 [DU], *Supporting Information*, for trajectory averages).

We calculate the normalized scalar product D between the vectors formed by the side chains of Val24 and Lys28. By comparing results on D (Fig. 5, *Supporting Information*) with Fig. 1, we observe a persistence of the relative orientation of the side chains of Val24 and Lys28 during $R^*(4,8)$ events. These rather stable values for D indicate that during $R^*(4,8)$ events the side chains of Val24 and Lys28 are constrained in their relative orientation by being bound to atoms in the monomer, increasing packing and possibly providing stability to the hydrophobic interaction. Trajectory [DU], however, does not show the same persistence in D values, suggesting that the lack of $R^*(4,8)$ and $R^*(2,8)$ events permit an unconstrained motion of the Val24 and Lys28 side chains.

Hydrogen Bonds. An examination of the number of HBs within the decapeptide as a function of time (Fig. 6 (upper), *Supporting Information*) reveals that only a handful of short-lived HBs are active during events. In SB events, there is one HB formed between one of the Glu22(O_ϵ) (or Asp23(O_δ)) and one of the Lys28(H_ζ). An exception is trajectory [RCS], in which the two SBs are present at the same time. The hydrophobic events ($R^*(4,8)$ and S^*), on the other hand, are not characterized by unique HBs, but consist instead of different HBs. In Fig. 3, we show contact maps where the strength of contacts is proportional to the number of HBs between two amino acids. In most trajectories, Lys28 is the most active amino acid in HB formation. In trajectory [RCS], the strongest contacts are the salt bridges between Glu22, Asp23, and Lys28.

By analyzing the frequency of formation of individual HBs during hydrophobic events (Table 4, *Supporting Information*), we determine that the highest percentage of HBs is formed between atoms belonging to the backbone. The most prevalent backbone HBs are Lys28(O)-Gly25(H) and Asp23(O)-Lys28(H), and in trajectory [RC] the longest hydrophobic event that is stabilized by the Lys28(O)-Gly25(H) also shows the tightest (smallest) loop formation. The NH_3^+ group of Lys28 also participate in SB formation (Table 4, *Supporting Information*) which is consistent with experimental results indicating that Lys28 in wild type $A\beta_{21-30}$ is protected from hydrogen exchange [32]. These results suggest that although transient backbone HBs may help stabilize an already existing loop, loop formation and long-term stability might lie in the hydrophobic interactions of Val24 and Lys28 rather than on specific HBs. On the other hand, loop formation in trajectory [RCS] is not only driven by hydrophobic interactions but, to a large extent, by SB interactions, suggested by the large number of SBs in comparison to backbone HBs (Table 4, *Supporting Information*).

4 Discussion

In this work, we present a series of all-atom MD simulations of a decapeptide folding nucleus of $A\beta$ monomer ($A\beta_{21-30}$) in explicit water and in water with addition of salt ions. We also present all-atom MD simulations of the Dutch $[Gln22]A\beta_{21-30}$ peptide. Our results indicate that for all five trajectories studied (four for wild type and one for Dutch), hydrophobic events, characterized by packing of the isopropyl group of Val24 and the butyl group of Lys28, predominate over electrostatic events (SB between Glu22 and Lys28 or between Asp23 and Lys28).

The hydrophobic events are highly correlated with a smaller value for the radius of

gyration R_g, and they last longer than SB events (Tables 1 and 2). In wild type $A\beta_{21-30}$ in normal density water, results on the distance between termini and lifetimes of backbone HBs indicate the formation of a semi-rigid loop in the Val24–Lys28 region with highly flexible termini, in close agreement with DMD studies [34] and consistent with NMR experiments of $A\beta_{21-30}$ [32].

In wild type $A\beta_{21-30}$ in reduced density water, the Val24–Lys28 region also forms a loop that during periods of time may belong to a larger helical structure (mostly π- and α-helices). The formation of a helix in our [P1] and [P2] trajectories is not surprising in light of prior studies of $A\beta$ and other proteins that form helices when the density of water is effectively reduced by other solvated components [12–15, 45]. The formation of helices in reduced density water is consistent with the possibility that hydrophobic assembly in proteins is facilitated by removal of water molecules ("vaporization") from regions between hydrophobic amino acids [46]. Future work in this area will have relevance for understanding how $A\beta$ folding is affected by different intra- and extracellular milieus, including endoplasmic and plasma cell membranes, endosomal/lysosomal compartments, cytoplasm, plasma, and cerebrospinal fluid.

Although in our trajectories with pure normal water the Glu22-Lys28 and Asp23-Lys28 salt bridges are transient in time, they could be precursors to the salt bridges proposed in recent molecular dynamics simulations [47] and modeling based on NMR data [48], where it is suggested that the turn or bend in $A\beta$ fibrils is stabilized by a salt bridge involving Asp23-Lys28. In Lazo *et al.* [32], however, the R(2,8) and R(3,8) distances (larger than 9Å) seem to rule out the existence of the SBs seen here. This finding can be reconciled by considering that NMR experiments measure averages of these distances and by differences in time scales, since the simulations here are done in the nanosecond time scale whereas NMR

experiments are performed in the microsecond to millisecond time scales.

In wild type $A\beta_{21-30}$ in normal water with solvated salt ions [RCS], we observe the longest "waiting time" for a hydrophobic event which can be attributed to the well-known salting-in effect in which a cloud of ions increases solubility of a protein by lowering its electrostatic free energy [49]. In this same trajectory, the coexistence of the Glu22-Lys28 and Asp23-Lys28 SBs during the $R^*(4,8)$ event might be a consequence of a disruption of the HB network of the water by the solvated ions. In this scenario, ions locally reorient water molecules, changing the HB interactions between water and Glu22 and Asp23, enhancing the simultaneous formation of these salt bridges.

In the [DU] trajectory, the lack of hydrophobic and SB events, and the unconstrained motion of the Val24 and Lys28 side chains, indicate a more flexible structure than the wild type, in agreement with [50]. Taken as a whole, results on the [DU] trajectory suggest that the Dutch mutation alters the folding pathway of the peptide. According to a postulate of Lazo *et al.* [32], partial unfolding of the Ala21–Ala30 region of $A\beta$ should occur prior to fibril formation. If this is so, then our observation of the inability of the [DU] trajectory to form a stable Val24-Lys28 loop might explain the higher propensity of the Dutch peptide to form protofibrils and fibrils.

5 Conclusions

Our results show that hydrophobic interactions play a crucial role in the $A\beta_{21-30}$ folding dynamics, assisted by the formation of salt bridges between the charged amino acids. By performing secondary structure and hydrogen bond analysis, we find that there is no regular secondary structure or permanent hydrogen bonding, suggesting that folding involves

formation of a loop stabilized by the packing of the side chains of Val24 and Lys28. We also show that by reducing the density of water we may induce formation of a π-helix. Interestingly, we find that in normal density water, if the solvent contains desolvated ions, the salt bridges play a prominent role in the stabilization of the Val24–Lys28 loop. Finally, we find that for the Dutch [Gln22]Aβ_{21-30} decapeptide in water, elimination of charge at position 22 disrupts the natural tendency of the monomer to form a long-lived Val24–Lys28 loop. This substitution likely alters the Aβ folding pathway, leading to the formation of alternative turn structures, including those stabilized solely by an Asp23–Lys28 salt bridge.

Acknowledgments

References

[1] Pike, C. J, Walencewicz, A. J, Glabe, C. G, & Cotman, C. W. (1991) *Brain Res.* **563**, 311–314.

[2] Hardy, J & Allsop, D. (1991) *Trends Pharmacol. Sci.* **12**, 383–388.

[3] Walsh, D. M, Lomakin, A, Benedek, G. B, Condron, M. M, & Teplow, D. B. (1997) *J. Biol. Chem.* **272**, 22364–22372.

[4] Harper, J. D, Wong, S. S, Lieber, C. M, & Lansbury, P. T. (1997) *Chem. Biol.* **4**, 119–125.

[5] Lambert, M. P, Barlow, A. K, Chromy, B. A, Edwards, C, Freed, R, Liosatos, M, Morgan, T. E, Rozovsky, I, Trommer, B, Viola, K. L, Wals, P, Zhang, C, Finch, C. E, Krafft, G. A, & Klein, W. L. (1998) *Proc. Natl. Acad. Sci. USA* **95**, 6448–6453.

[6] Klein, W. L, Krafft, G. A, & Finch, C. E. (2001) *Trends Neurosci.* **24**, 219–224.

[7] Yong, W, Lomakin, A, Kirkitadze, M. D, Teplow, D. B, Chen, S. H, & Benedek, G. B. (2002) *Proc. Natl. Acad. Sci. USA* **99**, 150–154.

[8] Walsh, D. M, Klyubin, I, Fadeeva, J. V, Cullen, W. K, Anwyl, R, Wolfe, M. S, Rowan, M. J, & Selkoe, D. J. (2002) *Nature* **416**, 535–539.

[9] Kirkitadze, M. D, Bitan, G, & Teplow, D. B. (2002) *J. Neurosci. Res.* **69**, 567–577.

[10] Klein, W. L, Stine Jr., W. B, & Teplow, D. B. (2004) *Neurobiol. Aging* **25**, 569–580.

[11] Barrow, C. J, Yasuda, A, Kenny, P. T. M, & Zagorski, M. (1992) *J. Mol. Biol.* **225**, 1075–1093.

[12] Sticht, H, Bayer, P, Willbold, D, Dames, S, Hilbich, C, Beyreuther, K, Frank, R. W, & Rosch, P. (1995) *Eur. J. Biochem.* **233**, 293–298.

[13] Coles, M, Bicknell, W, Watson, A. A, Fairlie, D. P, & Craik, D. J. (1998) *Biochemistry* **37**, 11064–11077.

[14] Shao, H. Y, Jao, S. C, Ma, K, & Zagorski, M. G. (1999) *J. Mol. Biol.* **285**, 755–773.

[15] Crescenzi, O, Tomaselli, S, Guerrini, R, Salvadori, S, D'Ursi, A. M, Temussi, P. A, & Picone, D. (2002) *Eur. J. Biochem.* **269**, 5642–5648.

[16] Zhang, S, Iwata, K, Lachenmann, M. J, Peng, J. W, Li, S, Stimson, E. R, Lu, Y, Felix, A. M, Maggio, J. E, & Lee, J. P. (2000) *J. Struct. Biol.* **130**, 130–141.

[17] Gursky, O & Aleshkov, S. (2000) *BBA - Protein Struct. M.* **1476**, 93–102.

[18] Lee, J. P, Stimson, E. R, Ghilardi, J. R, Mantyh, P. W, Lu, Y. A, Felix, A. M, Llanos, W, Behbin, A, Cummings, M, Vancriekinge, M, Timms, W, & Maggio, J. E. (1995) *Biochemistry* **34**, 5191–5200.

[19] Narayanan, S & Reif, B. (2005) *Biochemistry* **44**, 1444–1452.

[20] Klimov, D. K, Straub, J. E, & Thirumalai, D. (2004) *Proc. Natl. Acad. Sci. USA* **101**, 14760–14765.

[21] Bitan, G, Kirkitadze, M. D, Lomakin, A, Vollers, S. S, Benedek, G. B, & Teplow, D. B. (2003) *Proc. Natl. Acad. Sci. USA* **100**, 330–335.

[22] Bitan, G, Vollers, S. S, & Teplow, D. B. (2003) *J. Biol. Chem.* **278**, 34882–34889.

[23] Bitan, G, Tarus, B, Vollers, S. S, Lashuel, H. A, Condron, M. M, Straub, J. E, & Teplow, D. B. (2003) *J. Am. Chem. Soc.* **125**, 15359–15365.

[24] Urbanc, B, Cruz, L, Ding, F, Sammond, D, Khare, S, Buldyrev, S. V, Stanley, H. E, & Dokholyan, N. V. (2004) *Biophys. J.* **87**, 2310–2321.

[25] Urbanc, B, Cruz, L, Yun, S, Ding, F, Buldyrev, S. V, Bitan, G, Teplow, D. B, & Stanley, H. E. (2004) *Proc. Natl. Acad. Sci. USA* **101**, 17345–17350.

[26] Smith, S. W, Hall, C. K, & Freeman, B. D. (1997) *J. Comput. Phys.* **134**, 16–30.

[27] Dokholyan, N. V, Buldyrev, S. V, Stanley, H. E, & Shakhnovich, E. I. (1998) *Folding and Design* **3**, 577–587.

[28] Dokholyan, N. V, Buldyrev, S. V, Stanley, H. E, & Shakhnovich, E. I. (2000) *J. Mol. Biol.* **296**, 1183–1188.

[29] Smith, A. V & Hall, C. K. (2001) *Proteins Struct. Funct.* **44**, 344–360.

[30] Xu, Y, Shen, J, Luo, X, Zhu, W, Chen, K, Ma, J, & Jiang, H. (2005) *Proc. Natl. Acad. Sci. USA* **102**, 5403–5407.

[31] Massi, F & Straub, J. E. (2003) *J Comput Chem* **24**, 143–153.

[32] Lazo, N. D, Grant, M. A, Condron, M. C, Rigby, A. C, & Teplow, D. B. (2005) *Prot. Sci.* **14**, 1581–1596.

[33] Fersht, A. (1999) *Structure and Mechanism in Protein Science: a Guide to Enzyme Catalysis and Protein Folding.* (W. H. Freeman and Co., New York).

[34] Borreguero, J. M, Urbanc, B, Lazo, N, Buldyrev, S. V, Teplow, D. B, & Stanley, H. E. (2005) *Proc. Natl. Acad. Sci. USA* **102**, 6015–6020.

[35] Miravalle, L, Tokuda, T, Chiarle, R, Giaccone, G, Bugiani, O, Tagliavini, F, Frangione, B, & Ghiso, J. (2000) *J. Biol. Chem.* **275**, 27110–27116.

[36] MacKerell, J. A. D, Bashford, D, Bellott, M, L., D. J. R, Evanseck, J. D, Field, M. J, Fischer, S, Gao, J, Guo, H, Ha, S, Joseph McCarthy, D, Kuchnir, L, Kuczera, K, Lau, F. T. K, Mattos, C, Michnick, S, Ngo, T, Nguyen, D. T, Prodhom, B, Reiher, III, W. E, Roux, B, Schlenkrich, M, Smith, J. C, Stote, R, Straub, J, Watanabe, M, Wiorkiewicz Kuczera, J, Yin, D, & Karplus, M. (1998) *J. Phys. Chem. B* **102**, 3586–3616.

[37] Kalé, L, Skeel, R, Bhandarkar, M, Brunner, R, Gursoy, A, Krawetz, N, Phillips, J, Shinozaki, A, Varadarajan, K. , & K., S. (1999) *J. Comp. Phys.* **151**, 283–312.

[38] Jorgensen, W. L, Chandrasekhar, J, Madura, J. D, Impey, R. W, & Klein, M. L. (1982) *J. Chem. Phys.* **79**, 926–935.

[39] Levy, E, Carman, M. D, Fernandez Madrid, I. J, Power, M. D, Lieberburg, I, Van Duinen, S. G, Bots, G. T. A. M, Luyendijk, W, & Frangione, B. (1990) *Science* **248**, 1124–1126.

[40] Humphrey, W, Dalke, A, & Schulten, K. (1996) *J. Mol. Graphics* **14**, 33–38.

[41] Darden, T. A, York, D. M, & Pedersen, L. G. (1993) *J. Chem. Phys.* **98**, 10089–10092.

[42] Sanner, M. F, Olson, A. J, & Spehner, J. (1996) *Biopolymers* **38**, 305–320.

[43] Frishman, D & Argos, P. (1995) *Proteins Struct. Funct.* **23**, 566–579.

[44] Marqusee, S & Baldwin, R. L. (1987) *Proc. Natl. Acad.Sci. USA* **84**, 8898–8902.

[45] Fezoui, Y & Teplow, D. B. (2002) *J. Biol. Chem.* **277**, 36948–36954.

[46] Chandler, D. (2002) *Nature* **417**, 491–491.

[47] Ma, B. Y & Nussinov, R. (2002) *Proc. Natl. Acad. Sci. USA* **99**, 14126–14131.

[48] Petkova, A. T, Ishii, Y, Balbach, J. J, Antzutkin, O. N, Leapman, R. D, Delaglio, F, & Tycko, R. (2002) *Proc. Natl. Acad. Sci. USA* **99**, 16742–16747.

[49] Creighton, T. (1993) *Proteins: Structures and Molecular Properties, Second Edition.* (W. H. Freeman and Co., New York).

[50] Massi, F & Straub, J. E. (2001) *Biophys. J.* **81**, 697–709.

Figure Captions.

Figure 1. Distances between C_α atoms of Val24 and Lys28 (R(4,8)), charged atoms of Glu22-Lys28 (R(2,8), black), Asp23-Lys28 (R(3,8), gray), and radius of gyration R_g as a function of time for each trajectory. All of the distances are measured in Å.

Figure 2. Secondary structure of each amino acid as a function of time for each of the trajectories. The amino acid numbering is sequential from 21 (Ala21) to 30 (Ala30). The colors in the graphs correspond to: turn (green), bridge (tan), α-helix (pink), π-helix (red), and coil (white - none of the above).

Figure 3. Hydrogen bond contact maps for each of the trajectories. The level of darkness of each contact is proportional to the number of HBs between two amino acids (from Table 4, *Supporting Information*). The grayscale values for each contact map are relative to the maximum and minimum number of HBs from each trajectory. The strongest contacts per trajectory are: in [RC] between the Gly25 and Lys28; in [P1] between the Asp23 and Lys28; in [P2] between the Val24 and Gly29; in [DU] between the Gly25 and Lys28; and in [RCS] between the Glu22 and Lys28, and Asp23 and Lys28.

Table 1:

Table 2:

Figure 1:

Figure 2:

Figure 3: